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Evolution

In vivo rearrangement of mitochondrial DNA in *Saccharomyces cerevisiae*

(subgenomic recombination/dispensable DNA/recombinogenic sequences)

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ABSTRACT **A revertant (SPR1) from a high-frequency petite strain of *Saccharomyces cerevisiae* has been shown by mapping and sequence analysis to have a rearranged mitochondrial genome. *In vivo* rearrangement has occurred through a subgenomic-recombination pathway involving the initial formation of subgenomic molecules in nascent petite mutants, recombination between these molecules to form an intermediate with direct repeats, and subsequent excision of the resident or symposed duplication to yield a molecule with three novel junctions and a changed gene order. Sequencing of the novel junctions shows that intramolecular recombination in each case occurs by means of G+C-rich short direct repeats of 40–51 base pairs. Mapping and sequence analysis also reveal that the SPR1 mitochondrial genome lacks three sectors of the wild-type molecule of 4.4, 1.7, and 0.5 kilobases. Each of these sectors occurs in nontemplate, base-biased DNA, that is over 90% A+T. Absence of these sectors together with a rearranged gene order does not appear to affect the phenotype of SPR1, as colony morphology and growth rate on a number of different substrates are not detectably different from the wild type. Lack of phenotypic change suggests that mitochondrial gene expression has not been noticeably disrupted in SPR1 despite deletion of the consensus nonomer promoter upstream from the glutamic acid tRNA gene. Dispensability of DNA sectors and the presence of recombinogenic short, direct repeats are mandatory features of the subgenomic-recombination pathway for creating rearrangements in baker's yeast mtDNA. It is proposed that, in other organisms, organelle genomes containing these elements may undergo rearrangement by the same steps.**

Mapping of mitochondrial genomes in yeasts has revealed that both rearrangements and length mutations are widespread (1). Indeed, such changes are prevalent in mitochondrial genomes of lower eukaryotes (2) and flowering plants (3, 4). Moreover, chloroplast genomes of algae and some higher plants also show variability in size and gene order (5, 6). These observations raise questions as to how structural changes occur and whether alterations have any consequences for organelle gene expression.

Conceptually, rearrangements in circular DNA could occur by means of an intermediate molecule with a direct duplication. Loss of the resident or symposed duplication would lead to a rearrangement, whereas deletion of the foreign-placed or alloposed repeat would restore the original gene order. Formation of an intermediate molecule with direct duplications could take place in one of two ways. In the first instance, a subgenomic fragment could be inserted into the wild-type molecule to give a repeat (transposition). In the second case, two subgenomic molecules sharing a common segment could recombine in this region (subgenomic recombination) to form the intermediate. A mandatory feature of this second pathway is that the two subgenomic species must each lack a common part of the wild-type molecule [in specific terms, 4.4 kilobases (kb) of wild-type mtDNA, Fig. 1]. This ensures *a priori* that the resulting intermediate cannot return to the original molecule. Alternatively, in the case where two subgenomic species have two separate regions in common, it can be demonstrated diagrammatically that a resulting cointegrated intermediate could undergo recombination to restore the wild-type genome.

As stated above, a special facet of the subgenomic-recombination pathway is that the original wild-type molecule must contain at least one segment that is dispensable. Potentially dispensable sectors of yeast mtDNA are the base-biased regions that comprise over half the 80-kb molecule (2, 7). These regions, up to 7 kb long in one instance, are greater than 90% A+T. Interspersed in these stretches of A+T are various G+C "clusters" of 20–50 base pairs (bp) (8). As described below, three dispensable base-biased sectors and six G+C clusters are involved in the creation of a rearranged mitochondrial genome.

Formation of a mitochondrial genome containing direct duplications has been inferred from studies on high-frequency petite (hfp)-forming strains of *Saccharomyces cerevisiae* (9, 10). These respiratory competent strains have been obtained by mating nascent spontaneous petites (11). Characterization of mtDNA in hfp strains has suggested that the mitochondrial genome is composed of two subgenomic molecules (originating in the nascent petites) in equilibrium with a cointegrate (10). However, due to the large number of petite mutants in cultures of hfp strains (greater than 60%), it has not been possible to demonstrate directly the presence of the cointegrated form. Nevertheless, in the present study, sequence analysis of three novel junctions in mtDNA from an hfp revertant has shown that the rearranged mitochondrial genome has lost *three* sectors of the wild-type mtDNA. The loss of three sectors is strong support for the idea, first raised in an earlier report (12), that the rearrangement has been produced by means of the subgenomic-recombination pathway involving an intermediate molecule with a direct repeat.

MATERIALS AND METHODS

Yeast Strain and Media. Derivation of hfp strain 15.19S of *S. cerevisiae* has been described (9). GlyYP medium contains 2% (wt/vol) glycerol, 0.5% (wt/vol) Difco yeast extract, and 1.0% (wt/vol) Difco proteose peptone. GGYP medium is GlyYP medium containing 0.1% (wt/vol) glucose.

Isolation of Revertants. Putative spontaneous revertants from the hfp phenotype having unsectored morphology were obtained after incubation of colonies on GlyYP plates at 30°C for 4 weeks. Induced revertants of independent origin were

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Abbreviations: hfp, high-frequency petite; njf, novel junction fragment(s).

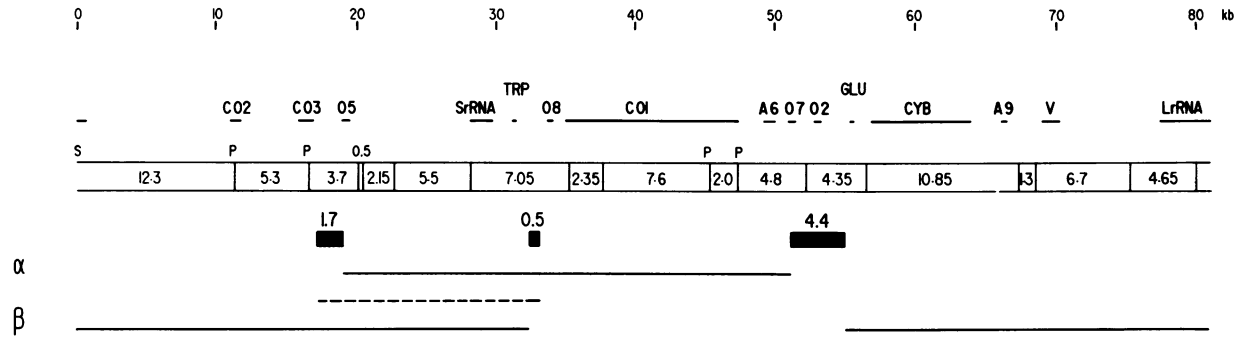

FIG. 1. Linearized map of wild-type *S. cerevisiae* mtDNA beginning at a unique *Sal* I (S) site in the gene encoding the large subunit of rRNA. The open boxes show the position and size of fragments generated by *Cfo* I–*Pvu* II digestion. P, *Pvu* II. For clarity, the 0.3- and 0.25-kb *Cfo* I fragments are shown as a single 0.5-kb fragment. Above the map is the position of the large genes: CO1–CO3, subunits 1–3 cytochrome oxidase; SrRNA and LrRNA, small and large subunits of rRNA; A6 and A9, ATPase subunits 6 and 9; CYB, apo cytochrome *b*; V, variant 1 protein. Also shown are four of the eight *ori/rep* sequences (O2, O5, O7, and O8) and genes encoding tRNAs for glutamic acid (GLU) and tryptophan (TRP). Filled boxes below the map show positions and sizes (in kb) of three regions missing from the rearranged SPR1 mtDNA. Lines labeled α and β show the retained segments in mtDNA from 15.19S. The dashed line shows the extent of the deletion that has occurred in the formation of the SPR1 mtDNA.

obtained as unsectored outgrowths from colonies growing on GlyYP plates containing ethidium bromide at 0.8 μg/ml.

Characterization of mtDNA. Total DNA was prepared from strains grown in GlyYP medium at 30°C by sodium dodecyl sulfate lysis of spheroplasts produced by zymolyase (13). Isolation of mtDNA from total DNA was by dye buoyant density centrifugation using bisbenzimide H33258 and CsCl (14). After digestion with restriction endonucleases *Cfo* I and *Pvu* II, DNA fragments were separated by electrophoresis in 1% agarose, transferred to a nylon membrane, and hybridized with ^{32}P-labeled DNA probes (Table 1). Labeling of probes, hybridization, and autoradiography have been described in detail elsewhere (14).

Cloning of mtDNA Fragments. Apart from pSCM11, all mtDNA fragments have been cloned into the phagemids pTZ18 or pTZ19 (Bio-Rad). Fragments to be cloned were cut from 1% agarose gels, extracted from agarose with Geneclean (Bio 101, La Jolla, CA), and ligated into the polysite region of the vectors with T4 DNA ligase. Transformation, identification of recombinant clones, and preparation of plasmid DNA have been described in detail (14).

Sequence Determination. Sequences were determined by the dideoxy chain termination procedure as described previously (14) except for the following changes: *Escherichia coli* Klenow polymerase was replaced with Sequenase (United States Biochemical). [α-^{35}S]dATP was used in place of the ^{32}P isotope, and 7-deaza-dGTP was substituted for dGTP to obtain sequence from G+C-rich palindromic regions. For sequence determination of the third novel junction that had been cloned into pUC13 (pSCM11), fragments were recloned into M13 as described previously (14). All other sequences were determined from single-stranded DNAs prepared directly from the pTZ18 and pTZ19 recombinant DNAs by superinfection of transformed cells with helper bacteriophage (15).

RESULTS

Isolation of Revertants. Revertants from the highly sectored 15.19S hfp phenotype were sought by screening for unsectored colonies. Only 2 spontaneous unsectored colonies were found among ≈4 × 10^4 sectored colonies. These isolates have spontaneous petite frequencies of ≈0.1% per generation (data not shown). Additional revertants were sought by treatment with ethidium bromide. Inclusion of ethidium bromide in plates decreased the number of respiratory competent colonies but did not lead to unsectored colonies among survivors. However, after 4 weeks at 30°C, some colonies showed smooth-margined outgrowing sectors that yielded nine isolates free from sectored colonies upon subculturing.

Table 1. Recombinant plasmids containing fragments of mtDNA

Plasmid	Source	Size of insert, bp	Description
pSCM11	SPR1	≈2000	*Alu* I–*Dra* I fragment cloned into the *Sma* I site of pUC13
pSCM12	WT	381	Subfragment from 1350-bp *Dra* I cloning (see pSCM27)
pSCM14	SPR1	135	Subfragment from 1900-bp *Dra* I cloning (see pSCM28)
pSCM17	WT	≈2400	*Eco*RI–*Cfo* I fragment blunt ended and cloned into the *Sma* I site of pTZ18
pSCM19	WT	≈850	*Pvu* II–*Dra* I fragment cloned into the *Sma* I site of pTZ18
pSCM20	WT	≈850	Fragment in opposite orientation to pSCM19
pSCM23	WT	≈1350	*Dra* I fragment cloned into the *Sma* I site of pTZ18
pSCM27	WT	≈1350	Fragment in opposite orientation to pSCM23
pSCM28	SPR1	≈1900	*Dra* I fragment cloned into the *Sma* I site of pTZ18
pSCM32	WT	≈600	Removal of ≈750 bp from pSCM27 by *Eco*RI–*Eco*RV cleavage, blunt ending, and religation
pSCM37	WT	≈900	*Hin*fI fragment cloned into the *Eco*RI site of pTZ19
pSCM38	WT	≈900	Fragment in opposite orientation to pSCM37
pSCM40	WT	≈1550	*Dra* I fragment cloned into the *Sma* I site of pTZ18
pSCM43	SPR1	≈700	*Hin*fI fragment cloned into the *Eco*RI site of pTZ18
pSCM44	WT	≈1550	Fragment in opposite orientation to pSCM40
pSCM45	SPR1	220	Removal of ≈480 bp from pSCM43 by *Sac* I–*Apa* I cleavage, blunt ending, and religation
pSCM46	WT	≈536	*Hin*fI–*Mbo* II fragment blunt ended and cloned into the *Sma* I site of pTZ18
pSCM47	WT	536	Fragment in opposite orientation to pSCM46

WT, wild type.

Examination of mtDNA in the two spontaneous and nine ethidium bromide-induced revertants revealed that all had the same change in their mtDNA (data not shown). Therefore the first isolated spontaneous revertant (SPR1) was chosen for a more detailed investigation.

Growth rates of SPR1 in both fermentable and nonfermentable substrates showed no detectable difference from wild type (data not shown), and colony morphology did not differ from wild type on solid medium containing glucose, galactose, glycerol, ethanol, or acetate.

Location of Three Novel Junctions in Rearranged mtDNA. In the first instance, the approximate location of the three novel junctions in SPR1 mtDNA was obtained by using probes for genic sequences (data not shown). Subsequently, positions of novel junctions were more accurately determined by using probes prepared from cloned fragments that were later used to determine sequences at the novel junctions. For the results presented in Fig. 3, probes were selected that lacked *ori/rep* sequences; otherwise, cross hybridization to many fragments occurred.

Positioning of the first novel junction was obtained by using two probes, pSCM14 and pSCM12, containing mtDNA fragments located adjacent to the 3′ end of ATPase 6 and downstream of *ori 5*, respectively (Fig. 2). With pSCM14, the wild-type 4.8-kb *Pvu* II–*Cfo* I band hybridizes, whereas in 15.19S and SPR1 a novel 4.9-kb *Pvu* II–*Cfo* II fragment is reactive (Fig. 3 and Table 2). This same fragment shows hybridization to pSCM12 in both 15.19S and SPR1 mtDNAs, whereas a 3.7-kb *Pvu* II–*Cfo* I fragment in both wild type and 15.19S shows labeling. The presence of two hybridizable bands in 15.19S mtDNA is consistent with the duplicated nature of part of this region in this genome (Fig. 1).

Location of the second novel junction was obtained by using probes from regions downstream of the tryptophan tRNA gene (pSCM45) and upstream of the glutamic acid tRNA gene (pSCM37, Fig. 2). In wild-type mtDNA, the 7.05-kb *Cfo* I–*Cfo* I fragment is labeled with pSCM45, whereas this fragment together with a novel 5.4-kb *Cfo* I band hybridizes in 15.19S mtDNA. In SPR1 mtDNA, the novel 5.4-kb fragment shows hybridization to pSCM45 as well as to pSCM37. This latter probe also labels the 5.4-kb band in 15.19S, whereas in wild type the 4.35-kb *Cfo* I–*Cfo* I fragment shows hybridization. Again the presence in 15.19S mtDNA of two bands hybridizing to the pSCM45 probe is consistent with the duplicated nature of part of this region in the hfp genome (Fig. 1).

Placement of the third novel junction was achieved with a probe from the 3′ end of the cytochrome oxidase subunit 3 gene (pSCM19) and a segment from upstream of *ori8* (680-bp fragment). With pSCM19, the 3.7-kb *Pvu* II–*Cfo* I wild-type band shows hybridization. This hybridizable fragment is also present in 15.19S mtDNA, whereas in SPR1 a novel 3.0-kb *Pvu* II–*Cfo* I band is labeled. This novel band also hybridizes with the 680-pb probe, whereas in wild type the 7.05-kb *Cfo* I–*Cfo* I fragment shows labeling. As the 680-bp probe also reacts weakly with other fragments, probably due to the presence of G+C clusters, the faint hybridization with the 7.05-kb fragment in the 15.19S mtDNA is barely apparent. However, labeling intensity of the 7.05-kb fragment in 15.19S mtDNA is comparable between the pSCM45 and 680-bp probes. A possible explanation for the low amount of this

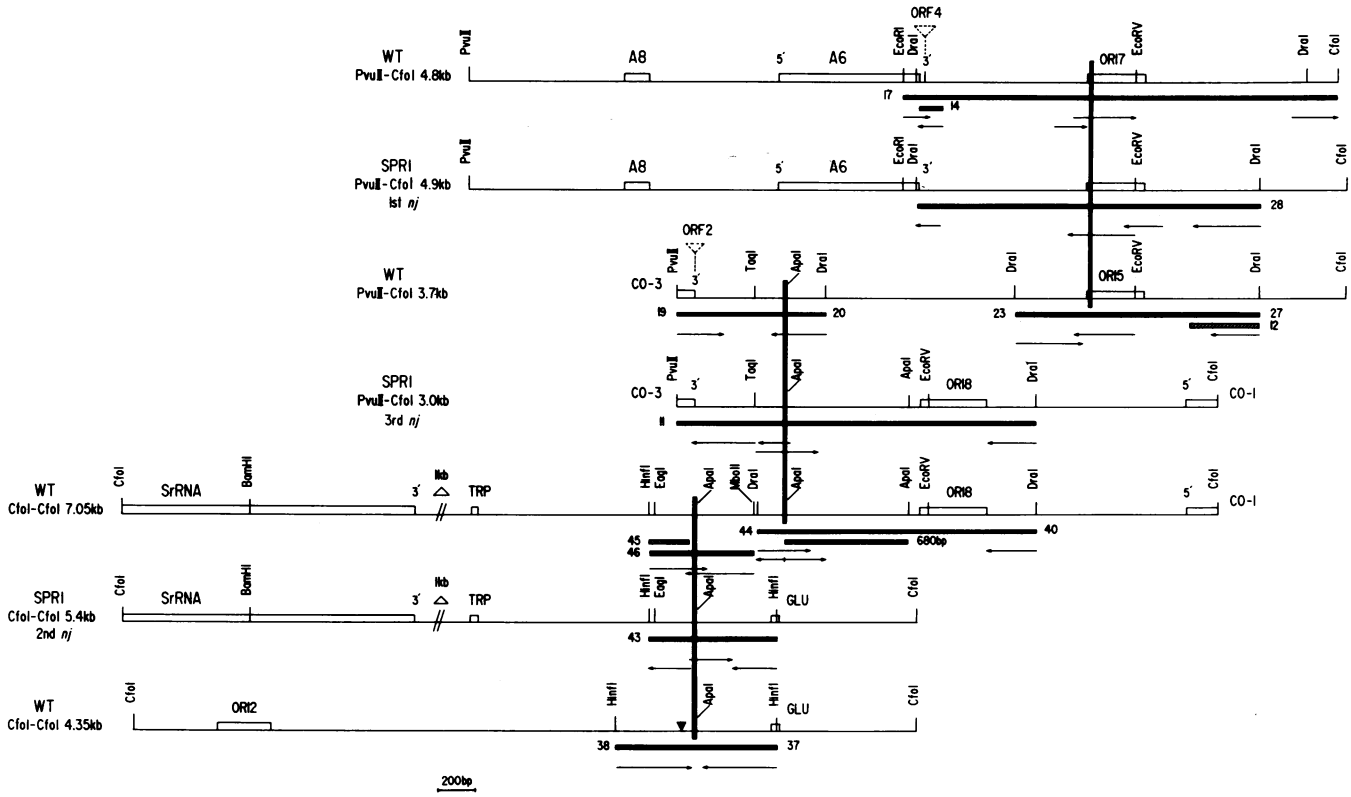

FIG. 2. Maps showing positions of cloned fragments and the sequencing strategy used in determining the three novel junctions (nj) in SPR1 mtDNA. Maps refer to the *Cfo* I–*Pvu* II restriction fragments illustrated in Figs. 1 and 3. Positions of landmark genes and other sequences are shown as open boxes and are identified as described in Figs. 1 and 3. Cloned fragments, numbered according to Table 1, are shown as filled or hatched horizontal boxes; the latter have also been used as probes (Table 1). Vertical lines show the position of the three novel junctions in SPR1 mtDNA and the positions of the deletion sites in wild-type (WT) mtDNA. The filled arrowhead on the bottom map shows the position of the consensus nonomer promoter upstream of the glutamic acid tRNA gene. The dotted triangles, labeled ORF2 and ORF4, refer to the positions of segments present in some wild-type mtDNAs but absent in the strain used in this study. The arrows below the map indicate the extent and direction of the determined sequences.

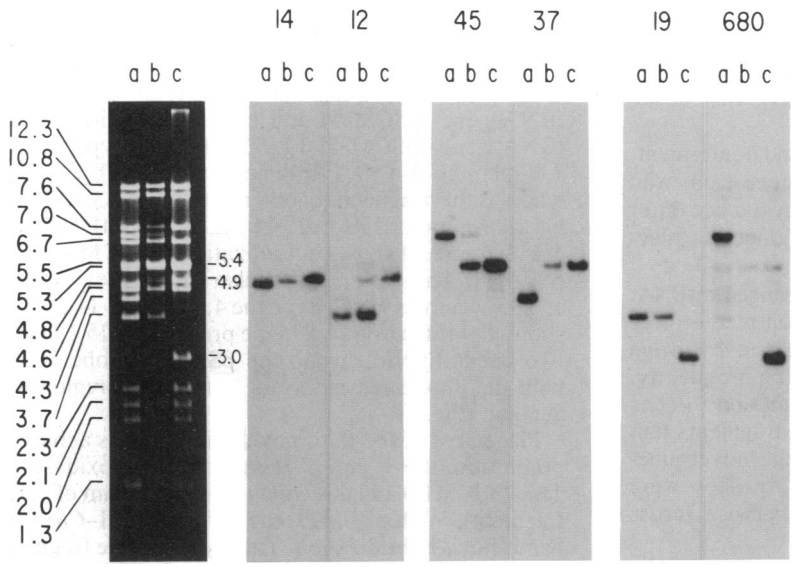

FIG. 3. Autoradiograms showing hybridization of ^{32}P-labeled mtDNA fragments (identified by pSCM numbers above the lane designations; see Table 1) to *Cfo* I–*Pvu* II digests of wild-type (lane a), 15.19S (lane b), and SPR1 (lane c) mtDNAs. The sizes of wild-type mtDNA fragments (in kb) are at the left while those of the three novel junction fragments are to the right. Filters have been grouped in pairs to illustrate common hybridization of two probes to the first (4.9 kb), second (5.4 kb), and third (3.0 kb) njf in the SPR1 mtDNA (lane c). The last filter (labeled 680) was hybridized with the 680-bp fragment upstream of *ori8* (Fig. 2, fifth map).

component in preparations of 15.19S mtDNA is that the majority of petites in the culture (≈90%; unpublished results) contain the β subgenomic molecule that lacks the 7.05-kb fragment (Fig. 1). The reason for the bias in favor of one type of defective genome in the petites in cultures of 15.19S is not understood.

In summary, these results show that in SPR1 mtDNA there are three novel bands that each hybridize to two separate probes. In the wild type, however, the pairs of probes hybridize to separate fragments (Table 2). In other words, parts of the genome that are separated in the wild-type molecule are now juxtaposed in the SPR1 mtDNA.

Sequence Determination of Three Novel Junctions. Characterization of the three novel junctions required a total of nine sequence determinations (six determinations from the wild type). Although >90% of *S. cerevisiae* mtDNA has been sequenced (7), gaps exist, and unfortunately gap 10A in the consensus sequence upstream of *ori8* is the location of two deletion sites. Because of this and other uncertainties associated with the use of a different strain, it became necessary to undertake six determinations from wild-type mtDNA in addition to analysis of the three novel junctions.

Table 2. *Cfo* I–*Pvu* II fragments from mtDNAs (kb)

Wild type	15.19S	SPR1
12.3	12.3	12.3
10.85	10.85	10.85
7.6	7.6	7.6
7.05	7.05	—
6.7	6.7	6.7
5.5	5.5*	5.5
—	5.4†	5.4†
5.3	5.3	5.3
—	4.9†	4.9†
4.8	—	—
4.65	4.65	4.65
4.35	—	—
3.7	3.7	—
—	—	3.0†
2.35	2.35	2.35
2.15	2.15*	2.15
2.0	2.0	2.0
1.3	1.3	1.3
0.3	0.3*	0.3
0.25	0.25*	0.25
Σ 81.15	90.5	74.55

Positions of restriction sites bounding the fragments are shown in Fig. 1. Fragments participating in the formation of njf are linked.
*Duplicated fragment.
†njf.

Isolation of novel junctions was greatly aided in two cases by the observation that *Dra* I digestion of SPR1 mtDNA produced two clearly resolved novel junction fragments (njf) of 2.8 and 1.9 kb corresponding to the third and first novel junctions, respectively (data not shown). Cloning of the 1.9-kb *Dra* I njf and a 2-kb *Pvu* II–*Dra* I subfragment of the 2.8-kb *Dra* I njf led to plasmids pSCM28 and pSCMII, respectively (Fig. 2 and Table 1). Isolation of the remaining (second) novel junction was achieved by using a two-step strategy. Survey experiments had established that a clearly resolved 3.5-kb *Bst*NI (*Fnu*DII) njf hybridized to a glutamic acid tRNA probe that also labeled an unresolved 700-bp njf in a *Hin*fI digest of SPR1 mtDNA. Hence plasmid pSCM43, containing the 700-bp *Hin*fI njf, was obtained by isolation of the 3.5-kb *Bst*NI fragment followed by cleavage of this with *Hin*fI.

In general, sequences were determined from each end of cloned fragments and, where required, use was made of unique restriction sites to delete segments of DNA so that internal sequences could be ascertained (Fig. 2). The results of this analysis are shown in Fig. 4, whereas the positions of the three novel junctions and the arrangement of genes on the circular map of SPR1 mtDNA are illustrated in Fig. 5.

DISCUSSION

Sequence analysis of the three novel junctions and surrounding regions of the SPR1 mitochondrial genome has established that this molecule is rearranged relative to the wild type. The first and second novel junctions have been formed by recombination by means of a directly repeated 40-bp G+C cluster of *ori7* and *ori5* and two copies of a 51-bp G+C cluster. Likewise deletion of the symposed repeat has proceeded by means of two other copies of a 49-bp G+C cluster. The involvement of G+C-rich repeats as preferential sites of recombination in mtDNA is in accord with observations from other studies (16, 17). Preference for G+C repeats is noteworthy because A+T repeats are more prevalent in base-biased sectors of mtDNA (18). A similar influence of base composition on deletion formation has been observed in bacteriophage T4, where G+C richness of direct repeats greatly stimulates deletion formation (19).

This study also demonstrates that three sectors of the wild-type mtDNA are lacking from the rearranged genome (Fig. 1). Loss of three sectors is strong evidence that the rearrangement has been generated by the subgenomic-

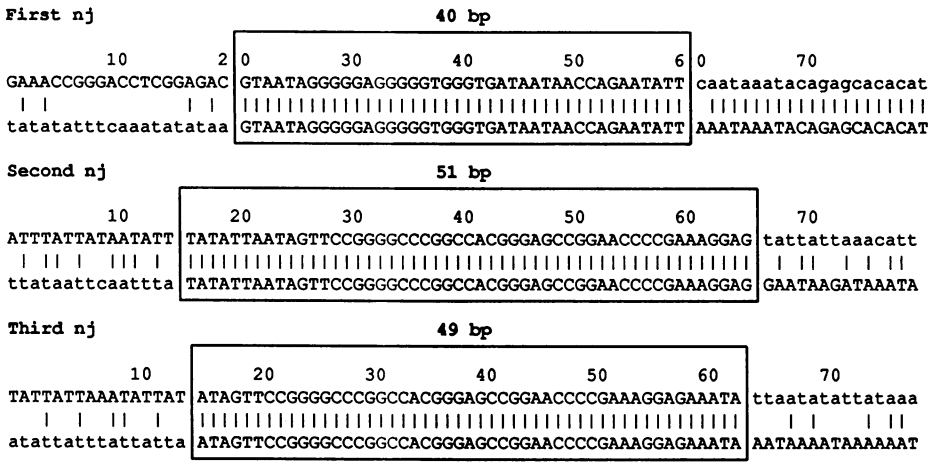

FIG. 4. DNA sequences of six wild-type regions involved in formation of the three novel junctions (nj). Sequences of the novel junctions are shown in uppercase letters including both sequences in the boxed region of the direct repeat. Although repeated sequences for the second and third novel junctions extend for 51 and 49 bp, respectively, they have a core of 44 bp in common corresponding to a G+C cluster named a1 (8).

recombination pathway. As stated previously, key facets of this pathway involve the initial production of subgenomic molecules that lack a common segment of the wild-type genome. Subsequently, a cointegrate containing direct repeats must be produced, at least transiently, to allow formation of the rearranged genome by excision of the symposed repeat and adjacent unique sequences (Figs. 1 and 5). Although in the present case deletion of the symposed repeat has included unique sequences at either end of the duplicated sector, it is not mandatory that this type of excision should occur in other revertants from hfp strains. Thus an imprecise deletion internal to the repeat may leave a small duplicated region.

Phenotypic changes resulting from the rearranged genome in SPR1 appear to be confined to a 5- to 10-fold decrease in spontaneous petite production. Furthermore, no changes in the growth rates of the SPR1 strain relative to wild type have been found in either fermentable or nonfermentable substrates. This observation is interesting on two counts: first, because it emphasizes the dispensable nature of the three missing sectors of the genome and second because the nonomer promoter upstream of the glutamic acid tRNA gene is no longer present. From knowledge on the location of promoters in wild-type mtDNA (20), it can be surmised that in SPR1 a transcript encompassing both the glutamic acid tRNA and cytochrome *b* genes must originate from the promoter for the rRNA small subunit sequence. However, processing of such a transcript would be expected to yield normal cytochrome *b* mRNA as the sequence downstream of glutamic acid tRNA, including the normal processing site, is unaltered (21). In other words, after processing, the cytochrome *b* mRNA in SPR1 would still contain the same 5′ leader region as in the wild type. No other known transcription units would be disrupted by the rearrangements.

Finally, it is apparent from the large amount of information on the structure of mtDNA in different *S. cerevisiae* strains that great plasticity exists in this genome with respect to optional introns (22) and optional base-biased sequences (8, 23). The present report extends our knowledge on dispensability of sectors and adds permutability to the list of variations tolerated by this genome.

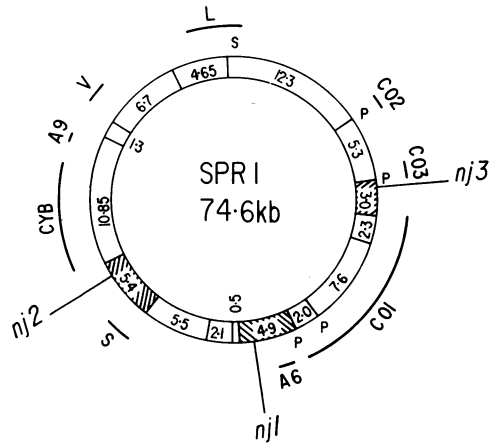

FIG. 5. Circular map for SPR1 mtDNA showing the position of the three novel junctions and three njf (hatched areas). Symbols used in identifying the landmark genes and sequences are as indicated in Fig. 1.

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1. Clark-Walker, G. D. (1985) in *The Evolution of Genome Size*, ed. Cavalier-Smith, T. (Wiley, Chichester, U.K.), pp. 277–297.
2. Wolf, K. & Del Giudice, L. (1988) *Adv. Genet.* **25**, 185–308.
3. Newton, K. J. (1988) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 503–532.
4. Levings, C. S., III, & Brown, G. G. (1989) *Cell* **56**, 171–179.
5. Palmer, J. D. (1985) *Annu. Rev. Genet.* **19**, 325–354.
6. Strauss, S. H., Palmer, J. D., Howe, G. T. & Duerksen, A. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3898–3902.
7. De Zamaroczy, M. & Bernardi, G. (1986) *Gene* **47**, 155–177.
8. De Zamaroczy, M. & Bernardi, G. (1986) *Gene* **41**, 1–22.
9. Evans, R. J., Oakley, K. M. & Clark-Walker, G. D. (1985) *Genetics* **111**, 389–402.
10. Evans, R. J. & Clark-Walker, G. D. (1985) *Genetics* **111**, 403–432.
11. Clark-Walker, G. D. & Miklos, G. L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 372–375.
12. Clark-Walker, G. D., Evans, R. J., Hoeben, P. & McArthur, C. R. (1985) in *Achievements and Perspectives of Mitochondrial Research II: Biogenesis*, eds. Quagliariello, E., Slatyer, E. C., Palmieri, F., Saccone, C. & Kroon, A. M. (Elsevier, Amsterdam), pp. 71–78.
13. Nasmyth, K. A. & Reed, S. I. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2119–2123.
14. Clark-Walker, G. D., McArthur, C. R. & Sriprakash, K. S. (1985) *EMBO J.* **4**, 465–473.
15. Cesareni, G. & Murray, J. A. H. (1987) *Genet. Eng.* **9**, 135–154.
16. Zinn, A. R., Pohlman, J. K., Perlman, P. S. & Butow, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2686–2690.
17. Dieckmann, C. L. & Gandy, B. (1987) *EMBO J.* **6**, 4197–4203.
18. De Zamaroczy, M. & Bernardi, G. (1987) *Gene* **54**, 1–22.
19. Singer, B. S. & Westlye, J. (1988) *J. Mol. Biol.* **202**, 233–243.
20. Tabak, H. F., Grivell, L. A. & Borst, P. (1983) *CRC Crit. Rev. Biochem.* **14**, 297–317.
21. Dieckmann, C. L., Koehner, T. J. & Tzagoloff, A. (1984) *J. Biol. Chem.* **259**, 4722–4731.
22. Dujon, B. (1981) in *The Molecular Biology of the Yeast Saccharomyces cerevisiae*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 505–635.
23. Sor, F. & Fukuhara, H. (1982) *Nucleid Acids Res.* **10**, 1625–1633.